Journal of Environmental Management 217 (2018) 478–485

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Deep exploitation of refractory organics in anaerobic dynamic membrane bioreactor for volatile fatty acids production from sludge fermentation: Performance and effect of protease catalysis

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ARTICLE INFO

Article history:
Received 11 February 2018
Received in revised form
20 March 2018
Accepted 22 March 2018

Keywords:
Protease catalysis
Waste activated sludge
Anaerobic fermentation
Dynamic membrane bioreactor
Refractory protein

ABSTRACT

Volatile fatty acids (VFAs) production from waste activated sludge fermentation could be improved in anaerobic dynamic membrane bioreactor (ADMBR) by retaining residual organics within the reactor and prolonging their reaction time. However, the accumulation of refractory organics made it operate unstably. Therefore, protease catalysis was adopted to deeply exploit those refractory organics in sludge. By combining with dynamic membrane retention, protease catalysis indeed presented a good performance. VFAs yield was further enhanced by over 40% in ADMBR. Membrane fouling was slightly relieved due to protein and polysaccharide degradations in the sludge of dynamic membrane. It was also interestingly found that not only protease activity of sludge was improved from 5 to 21 U/ml, but also β-GLC activity was enhanced from 13 to 20 μmoL/L/h. Microbial community analysis showed protease addition could reduce bacterial richness and evenness in sludge, and accelerate the growth of polysaccharides-hydrolyzing bacteria, as well as inhibit some proteolytic bacteria.

1. Introduction

In recent years, the amount of waste activated sludge (WAS) has been increasing in wastewater treatment plants (WWTPs) of China, and reached even 55 million tons with water content of 80% in 2015 (National Bureau of Statistics of People's Republic of China, 2016). Anaerobic fermentation for volatile fatty acids (VFAs) production provides a new alternative for WAS reduction and resource recovery. Especially, with the introduction of "carboxylate platform" (Agler et al., 2011), technologies of efficiently utilization of VFAs from sludge fermentation, such as high-added value long-chain fatty acids (Li et al., 2018; Cavalcante et al., 2017; Zhang et al., 2017) and fatty acid methyl ester (Wallis et al., 2017), have greatly stimulated the vigorous development of WAS fermentation for VFAs production.

Low conversion rate of organics in WAS, mainly caused by the residuals of proteins and polysaccharides, is the bottleneck restricting the application of fermentative VFAs production technology (Liu et al., 2013; He et al., 2018). About 60–70% of organic matters in WAS has the potential of being biologically utilized (Hao et al., 2014) while only 30–40% of them is finally converted into VFAs (Yin et al., 2016). Moreover, the organics remained in the residual sludge not only leads to the low efficiency of sludge anaerobic fermentation, but also influences the dewatering performance of the fermented sludge (Zhu et al., 2015). The introduction of dynamic membrane separation into conventional sludge fermenter could greatly improve the conversion efficiency of organics in sludge by prolonging substrates retention time and relieving products feedback inhibitions (Liu et al., 2016a). However, long-term operation showed that there were still a large amount of organic residues, especially refractory protein, accumulated in the ADMBR, which made its operation instable.

In order to improve the conversion rate of organics in WAS, previous attentions mainly focused on sludge pretreatment, but neglected the exploitation of those organic residues. However, by flask tests, Yin et al. (2016) proved those organic residues were indeed exploitable. There are already many kinds of methods for the improvement of organics conversion in WAS, such as thermal, alkaline, ultrasonic and oxidative hydrolyses (Han et al., 2017; Xiao

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https://doi.org/10.1016/j.jenvman.2018.03.103
0301-4797/

et al., 2015; Jochen et al., 2018; Marta et al., 2007), but most of which would cause serious lysis of functional bacteria and are not suitable for the on-site application in biological reactors. It was reported that biological enzyme hydrolysis had the characteristics of mild reaction condition, few by-products, low energy consumption and high efficiency during sludge pretreatment (Barjenbruch and Kopplow, 2003; Yu et al., 2013; Kim et al., 2013). Therefore, though there is no related reports yet, protease catalysis seems to be suitable for the online exploitation of the accumulated residuals in ADMBR.

Furthermore, by comparing with conventional anaerobic bioreactors, there are several superiorities that make protease catalysis more suitable to be applied on ADMBR. Firstly, refractory organics can be concentrated within reactor by the retention of dynamic membrane, which greatly improves the efficiency of protease catalysis and reduces the amount of protease consumption. Secondly, most of the externally added protease can be retained in the reactor by membrane separation, especially for those combining with macromolecule organics, which makes the added protease fully utilized and provides the possibility of stable operation for ADMBR. Finally, under the pressure of sludge cells disruption due to protease catalysis, membrane separation could help non-sensitive bacteria, such as bacteria with cuticle, to survive and flourish. In conventional reactors, those bacteria could be washed out along the discharging due to their slow growth rate.

Thus, the objective of this study is to accelerate the hydrolysis of the residual organics in ADMBR by on-site application of protease catalysis, and ensure its stable operation in aspects of VFAs production, membrane fouling and microbial maintain. The performance of protease catalysis in promoting VFA production and residual organics degradation were investigated, and its influences on membrane fouling and microbial hydrolase activities were analyzed. The sustainability of microbial ecosystems was evaluated according to diversity indices, taxonomic distribution and redundancy analysis (RDA).

2. Material and methods

2.1. ADMBR

The ADMBR used in this test was the same as the reactor reported in previous report (Liu et al., 2016a). The operation conditions were also adopted the optimal values obtained in previous experiments, including total hydraulic reaction time (HRT) of approximately 5.4 d (Ma et al., 2016), pH of around 10.0 (Liu et al., 2012; Feng et al., 2009) and normal temperature of about 37 °C. Also, dynamic membrane subassembly was used in the ADMBR, in which, silk with an aperture of approximately 75 μm was used as the separation layer. The long HRT made a low membrane flux of 1.0–1.5 ml/m^2·min feasible in the ADMBR, which greatly relieved membrane fouling. After sampling on the 8th day, the influent and effluent were stopped for 8 h, but the continuous stirring was kept to maintain stable cross-flow on membrane surface for online cleaning dynamic membrane. Then, the ADMBR was operated normally. Therefore, there are about 16 h for the re-forming of dynamic membrane before sampling on day 9, during this period, the missed feeding was replenished to keep the organic loading constant. Then, protease was added along with feeding on day 10.

2.2. Substrates

WAS used as the substrate in this study was sampled, pretreated and stored just as the same as that mentioned in the previous paper (Liu et al., 2016a). The WAS had a pH of 6.7 ± 0.3, a soluble COD (SCOD) of 0.9 ± 0.08 g/L, a soluble VFAs concentration of 0.75 ± 0.06 g/L, a total solids (TS) concentration of 15.3 ± 1.3 g/L, a volatile solids (VS) concentration of 6.3 ± 0.4 g/L, a soluble protein concentration of 1.1 ± 0.1 g/L, and a soluble reducing sugar concentration of 0.4 ± 0.04 g/L.

2.3. Seeding sludge for anaerobic fermentation

Fermented sludge from another stably operating ADMBR for sludge fermentation was collected as the seeding sludge in this test. It had a pH of 10.5 ± 0.3, a SCOD of 10.25 ± 0.15 g/L, a soluble VFAs concentration of 4.5 ± 0.4 g/L, a TS concentration of 15.25 ± 0.3 g/L, a VS concentration of 4.0 ± 1.0 g/L, a soluble protein concentration of 0.85 ± 0.25 g/L, and a soluble reducing sugar concentration of 0.55 ± 0.03 g/L.

2.4. Hydrolase

Hydrolase used in this test was commercial alkaline protease with activity of 80000 U/g (from Amano in Japan), and could kept activity in the pH range of 9–12. The theoretical value of microorganism protease activity in sludge contributed by externally protease addition (μ, U/ml) could be calculated out by the model 1.

$$\mu = D \times U \times TS \times 10^{-6} \quad (1)$$

where D is the addition dosage of the commercial alkaline protease (mg protease/g WAS), U is the activity of the commercial alkaline protease (U/g), and TS is the total solids in ADMBR (g/L).

2.5. Anaerobic fermentation

As enough reports had proved that alkaline fermentation of WAS presented higher VFAs production than those of neutral and acidic fermentations (Li et al., 2017; Zhao et al., 2018; Ma et al., 2016), WAS alkaline fermentation with pH of 10 was thus adopted in this study. The total process of sludge anaerobic fermentation could be divided into two stages, without (stage 1) and with (stage 2) protease addition. In stage 1, the fermenter was filled with WAS of 13 L and seeding sludge of 1.3 L. Dissolved oxygen in the WAS and the gas in the headspace of the flasks were removed by sparging gaseous nitrogen for approximately 30 min to maintain a strictly anaerobic condition. Then, pH was adjusted to 10.0, temperature was controlled at about 37 °C and stirring intensity was stabled at 100 r/min. During the period of batch operation in day 1–3, full effluent recycle was implemented to accelerate dynamic membrane formation, that is, all of the effluent was pumped into reactor again as the influent. As the experiment proceeded, VFAs concentration in fermenter increased. When the VFA concentration in reactor tended to be stable, continuous feeding and discharging were implemented. Then, keeping stable operation until that the VFA concentration in the effluent reached stable, stage 1 ended and stage 2 began. In stage 2, operation parameters of TS, temperature, pH, HRT and stirring intensity, were kept constantly, while protease with dosage of 25 mg/g dry sludge (DS) was added along with the feeding. The protease dosages of 0, 5, 10, 15, 20, 25 and 30 mg/g DS had been tested by beaker experiments and 25 mg/g DS was found to be the optimum. Samples were removed from fermenter and analyzed at intervals of 24 h.

2.6. Analytical methods

2.6.1. Measurements of conventional indexes

Conventional indices, including pH, COD, VS, TS and VFA, as well as soluble carbohydrate and protein concentrations were measured according to those methods mentioned in previous paper (Liu et al.,